

CLAIMS

1. An isolated polynucleotide encoding a mosquito deoxyribonucleoside kinase derived from a yellow fever mosquito, said isolated polynucleotide being selected from the group consisting of:
- a. an isolated polynucleotide encoding multisubstrate deoxyribonucleoside kinase derived from yellow fever mosquito *Aedes aegypti*,
 - b. an isolated polynucleotide having the nucleotide sequence of SEQ ID No. 1,
 - 10 c. an isolated polynucleotide encoding a polypeptide having the sequence of SEQ ID No. 2,
 - d. an isolated polynucleotide encoding a multisubstrate deoxyribonucleoside kinase, wherein said polynucleotide has at least 70% sequence identity to SEQ ID No. 1,
 - 15 e. an isolated polynucleotide encoding a multisubstrate deoxyribonucleoside kinase having at least 80% sequence identity to SEQ ID No. 2,
 - f. an isolated polynucleotide capable of hybridising to the complement of a polynucleotide having the nucleotide sequence of SEQ ID No. 1, said isolated polynucleotide encoding a multisubstrate deoxyribonucleoside kinase, and
 - 20 g. the complement of any of a through f.

2. The polynucleotide of claim 1, encoding a deoxyribonucleoside kinase enzyme derived from a *Aedes aegypti*, which kinase enzyme, when compared to human *Herpes simplex* virus 1 (HSV-TK1) and upon transformation into an eukaryotic cell, decreases at least four (4) fold the IC₅₀ of at least one nucleoside analogue, such as Gemcitabine or AraC.

3. The polynucleotide of claim 1, encoding a deoxyribonucleoside kinase variant derived from mosquito, which deoxyribonucleoside kinase enzyme variant, when compared to human *Herpes simplex* virus 1 (HSV-TK1) and upon transformation into an eukaryotic cell, decreases at least four (4) fold the IC₅₀ of at least one nucleoside analogue, such as Gemcitabine or AraC.

4. The polynucleotide of claim 1, wherein the isolated polynucleotide has the nucleotide sequence of SEQ ID No. 1.

5. The polynucleotide of claim 1, wherein the isolated polynucleotide encodes a polypeptide having the sequence of SEQ ID No. 2.

6. The polynucleotide of claim 1, wherein the isolated polynucleotide is capable of hybridising to the complement of a polynucleotide having the nucleotide sequence of SEQ ID No. 1, said isolated polynucleotide encoding a multisubstrate
5 deoxyribonucleoside kinase.

7. The isolated polynucleotide of claim 1, which has at least 73%, preferably at least 75%, more preferred at least 80%, more preferred at least 85%,
~~even more preferred at least 90%, yet more preferred at least 95%, most preferred at~~
10 least 98% identity to the polynucleotide sequence presented as SEQ ID NO: 1 when determined over its entire length.

8. The isolated polynucleotide of claim 1, encoding a multisubstrate deoxyribonucleoside kinase having at least 80% sequence identity to
15 SEQ ID No. 2, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98 %, when determined over the entire length of SEQ ID No. 2.

9. The isolated polynucleotide of claim 1, encoding a C-terminally
20 truncated multisubstrate deoxyribonucleoside kinase.

10. An isolated mosquito deoxyribonucleoside kinase enzyme selected from the group consisting of:

- 25 a. an isolated mosquito deoxyribonucleoside kinase enzyme encoded by the polynucleotide of any one of the claims 1-9,
- b. an isolated mosquito deoxyribonucleoside kinase enzyme derived from from yellow fever mosquito *Aedes aegypti*,
- c. a polypeptide having the sequence of SEQ ID No. 2, and
- 30 d. a multisubstrate deoxyribonucleoside kinase having at least 80% sequence identity to SEQ ID No. 2.

11. The isolated multisubstrate deoxyribonucleoside kinase of claim 7, being derived from yellow fever mosquito *Aedes aegypti*

35 12. The isolated deoxyribonucleoside kinase of claim 10, which multisubstrate deoxyribonucleoside kinase enzyme, when expressed and compared to human *Herpes simplex* virus 1 (HSV-TK1) and upon transduction into a eukaryotic cell, decreases at least four (4) fold the IC₅₀ of at least one nucleoside analogue, such as Gemcitabine or AraC.

13. The mosquito deoxyribonucleoside kinase of claim 10, comprising the amino acid sequence of SEQ ID NO: 2, or an amino acid sequence of at least 85%, more preferred at least 90%, yet more preferred at least 95%, most preferred at least 98% identity with this sequence, when determined over its entire length.

14. The mosquito deoxyribonucleoside kinase of claim 10 comprising the amino acid sequence of SEQ ID NO: 2, or a functional analogue thereof.

15. The mosquito deoxyribonucleoside kinase of any one of claims 10-14, which decreases at least three (3) fold the lethal dose (LD₁₀₀) of at least one nucleoside analogue when compared to the action of a thymidine kinase derived from human *Herpes simplex* virus 1 (HSV-TK1).

16. A vector construct comprising the polynucleotide of any of claims 1-9, and a promoter operably linked to the polynucleotide.

17. The vector construct of claim 16 being a viral vector, in particular a *Herpes simplex* viral vector, an adenoviral vector, an adenovirus-associated viral vector, a lentivirus vector, a retroviral vector or a vacciniaviral vector.

18. A packaging cell line capable of producing an infective virion comprising the vector of either of claims 16 to 17.

19. An isolated host cell genetically modified with the polynucleotide of any of claims 1-9, or the vector of either of claims 16-17.

20. The host cell of claim 19, which is a eukaryotic cell such as a human cell, a dog cell, a monkey cell, a rat cell or a mouse cell.

21. The host cell of claim 20, being selected from the group consisting of human stem cells, and human precursor cells, such as human neural stem or precursor cells, human hematopoietic stem or precursor cells, human hepatocyte progenitors, and mesenchymal stem cells.

22. The host cell of claim 19, which is a prokaryotic cell such as a bacterial cell, such as *E. coli*.

23. A pharmaceutical composition comprising the mosquito deoxyribonucleoside kinase enzyme of any of claims 10 to 15, and a pharmaceutically acceptable carrier or diluent.

5 24. A pharmaceutical composition comprising the polynucleotide of any of claims 1-9, or a vector of either of claims 16 and 17, and a pharmaceutically acceptable carrier or diluent.

10 ~~25. A pharmaceutical composition comprising the packaging cell line of~~
claim 18, or the host cell of either of claims 19 to 22, and a pharmaceutically acceptable carrier or diluent.

15 26. Articles containing a nucleoside analogue and a source of an *Aedes aegypti* derived deoxyribonucleoside kinase for the simultaneous, separate or successive administration in cancer therapy.

27. Articles according to claim 26, wherein the nucleoside analogue is a cytidine analogue.

20 28. Articles according to claim 26, wherein the nucleoside analogue is Gemcitabine or AraC, preferably Gemcitabine.

25 29. Articles according to claim 26, wherein the source of deoxyribonucleoside kinase comprises the nucleotide sequence of any of the claims 1 to 9 or the vector of either of claims 16 and 17.

30 30. Articles according to claim 26, wherein the source of deoxyribonucleoside kinase comprises the polypeptide of any of the claims 10 to 15.

31. Articles according to claim 26, wherein the source of deoxyribonucleoside kinase comprises the host cell of either of claims 19 to 22.

32. Articles according to claim 26, wherein the source of deoxyribonucleoside kinase comprises the packaging cell line of claim 18.

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33. A method of sensitising a cell to a nucleoside analogue prodrug, which method comprises the steps of

(i) transfecting or transducing said cell with a polynucleotide sequence according to any of claims 1 to 9 encoding a deoxyribonucleoside

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kinase enzyme, that promotes the conversion of said prodrug into a (cytotoxic) drug; and
(ii) delivering said nucleoside analogue prodrug to said cell;
wherein said cell is more sensitive to said (cytotoxic) drug than to said nucleoside
5 analogue prodrug.

34. The method of claim 33, wherein the nucleoside analogue is a cytidine analogue.

10 35. The method of claim 33, wherein the nucleoside analogue is gemcitabine (dFdC) or AraC, preferably gemcitabine.

36. A method of inhibiting a pathogenic agent in a warm-blooded animal, which method comprises administering to said animal a polynucleotide of any
15 of claims 1-9, or a vector of either of claims 16 to 17.

37. The method of claim 36, wherein said polynucleotide sequence or said vector is administered *in vivo*.

20 38. The method of either of claims 36-37, wherein said pathogenic agent is a virus, a bacteria or a parasite.

39. The method of either of claims 36-37, wherein said pathogenic agent is a tumour cell.
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40. The method of either of claims 36-37, wherein said pathogenic agent is an autoreactive immune cell.

41. The method of any of claims 36-40, further comprising the step
30 of administering a nucleoside analogue to said warm-blooded animal.

42. The method of claim 41, wherein said nucleoside analogue is a cytidine analogue.

35 43. The method of claim 41, wherein said nucleoside analogue is gemcitabine (dFdC), or AraC, preferably gemcitabine.

44. Use of the mosquito deoxyribonucleoside kinase enzyme of any of claims 10 to 15 for the phosphorylation of a nucleoside or a nucleoside analog.

45. The use of claim 44, wherein the nucleoside or nucleoside analog is a purine.

5. 46. A method of phosphorylating a nucleoside or a nucleoside analog, comprising the steps of
- i) subjecting the nucleoside or nucleoside analog to the action of the mosquito deoxyribonucleoside kinase enzyme of any of claims 10 to 15,
 - and
 - 10 ii) recovering the phosphorylated nucleoside or nucleoside analog.

47. The method of claim 46, wherein the nucleoside or nucleoside analog is a purine.

- 15 48. A method of non-invasive nuclear imaging of transgene expression of a mosquito deoxyribonucleoside kinase enzyme of the invention in a cell or subject, which method comprises the steps of
- (i) transfecting or transducing said cell or subject with a polynucleotide sequence encoding the mosquito deoxyribonucleoside kinase enzyme of any of the claims 10 to 15, which enzyme promotes the conversion of a substrate into a substrate-monophosphate;
 - (ii) delivering said substrate to said cell or subject; and
 - (iii) non-invasively monitoring the change to said prodrug in said cell or subject.

49. The method of claim 48, wherein the monitoring carried out in step (iii) is performed Single Photon Emission Computed Tomography (SPECT), by Positron Emission Tomography (PET), by Magnetic Resonance Spectroscopy (MRS), by Magnetic Resonance Imaging (MRI), or by Computed Axial X-ray Tomography (CAT), or a combination thereof.

50. The method of either of claims 48 to 49, wherein the substrate is a labelled nucleoside analogue.

51. Use of the nucleotide sequence according to any of claims 1 to 9 for the preparation of a medicament.

52. Use of the deoxyribonucleoside kinase enzyme according to any of the claims 10 to 15 for the preparation of a medicament.

53. Use of the expression vector of any of the claims 16 to 17, the isolated host cell of any of the claims 19 to 22 or the packaging cell line of claim 18 for the preparation of a medicament.

54. The use of any of claims 51 to 53, wherein the medicament is for the treatment of cancer.

55. A method of preparing the deoxyribonucleoside kinase enzyme of any of claims 10 to 15 comprising culturing a host cell according to any of claims 19 to 22 and recovering the enzyme from the culture medium and/or the cells.

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